## I. INTRODUCTION

A study was conducted to validate methods for the determination of residues of etofenprox and its α-CO and 4'-OH metabolites in soil, sediment and water. Untreated soil and sediment, collected from field dissipation and aquatic dissipation sites, respectively, were fortified at concentrations of 0.0100 and 0.100 mg/Kg with etofenprox, α-CO and 4'-OH followed by analysis to determine quantitative recoveries of the analytes. Untreated water, collected from an aquatic dissipation field site, was fortified at concentrations of 0.0500 and 0.500 µg/L with etofenprox, α-CO and 4'-OH followed by analysis to determine quantitative recoveries of the analytes. Samples were processed and analyzed for the determination of etofenprox, α-CO and 4'-OH using high performance liquid chromatography with mass spectral detection (LC/MS/MS) for final quantitation.

This study was conducted by Wildlife International, Ltd. according to the protocol "Analytical Method Validation for the Determination of Etofenprox, α-CO and 4'-OH in Soil, Water and Sediment", Appendix I, and was identified as Wildlife International, Ltd. Project Number 236C-135. Samples were prepared and analyzed from August 31 to September 23, 2005. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 236C-135 in archives located on the Wildlife International, Ltd. site.

## II. PURPOSE

This study was performed to validate methods for the determination of residues of etofenprox, α-CO and 4'-OH in soil, sediment and water. The methods were validated for subsequent application to analysis of soil, sediment and water collected to assess residue concentrations following application of formulated products containing etofenprox active ingredient in field dissipation and aquatic field dissipation studies.

## III. EXPERIMENTAL DESIGN

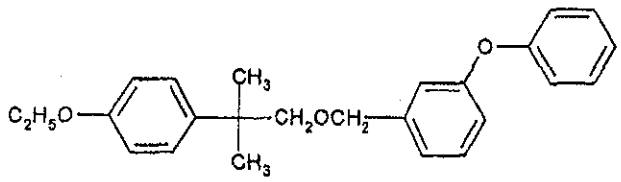
Water, soil and sediment were obtained from field sites selected for assessment of the dissipation of etofenprox and its metabolites α-CO and 4'-OH in aquatic and field dissipation studies. Soil, water and sediment were fortified with each analyte (etofenprox, α-CO and 4'-OH) at two different concentrations and analyzed according to the methods to be applied for quantitation of residues in field-collected samples from dissipation studies. Soil and sediment were fortified with each analyte, (etofenprox, α-CO and 4'-OH) at concentrations of 0.0100 and 0.100 mg/Kg. Water was fortified with each analyte (etofenprox, α-CO and 4'-OH) at concentrations of 0.0500 and 0.500 µg/L. The higher fortification concentration level for each substrate was selected in anticipation they would exceed the highest measured field residue. The lower concentration for each substrate was selected based on the anticipated practical limit of quantitation (LOQ) of the method. Reagent and matrix blanks (controls) for each analyte and substrate combination were analyzed concurrently to evaluate any potential analytical method interference.

## IV. MATERIALS AND METHODS

### A. Test Substances

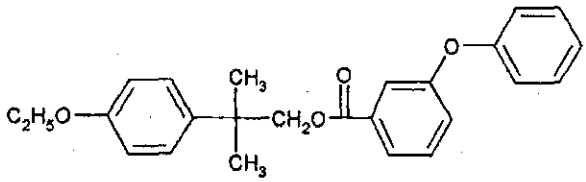
Test substances of etofenprox, the α-CO metabolite of etofenprox and the 4'-OH metabolite of etofenprox were received from Landis International, Inc. on April 13, 2005. These test substances were assigned Wildlife International, Ltd. Identification Numbers 7131, 7133 and 7132, respectively, and transferred to ambient storage in darkness. The etofenprox standard was subsequently transferred to refrigerated storage. Certificates of Analysis (Appendix II) were received with each test substance and provided the following information:

Etofenprox
ISO Name: Etofenprox
Test Name: MTI-500
CAS Number: 80844-07-1
Structural Formula:



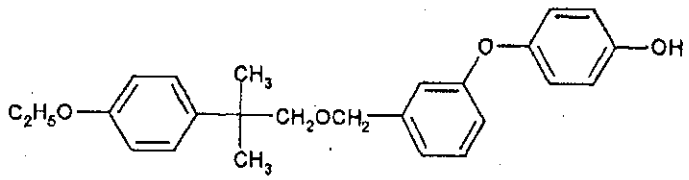

Molecular Weight: 376.47
Chemical Name: 2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether (IUPAC)
Appearance: White crystal
Lot Number: 9604
Purity: 99.9% by HPLC/UV (254 nm)
Expiration Date: December 2006

α-CO
Test Name: α-CO
Structural Formula:




Molecular Weight: 390.48
Chemical Name: 2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzoate
Appearance: White crystal
Lot Number: LS9911
Purity: 99.4% by HPLC/UV (254 nm)
Expiration Date: December 2008

4'-OH
Test Name: 4'-OH
Structural Formula:




Molecular Weight: 392.50
Chemical Name: 2-(4-ethoxyphenyl)-2-methylpropyl 3-(4-hydroxyphenoxy)benzyl ether
Appearance: White Crystals
Lot Number: 043-011222-1
Purity: 96.0% by HPLC/UV (254 nm)
Expiration Date: December 2008

B. Reagents and Solvents

All solvents used in this study were HPLC grade Burdick & Jackson, High Purity Solvent. Reagents were ACS reagent grade or equivalent quality. Sodium sulfate used in the sediment method was obtained from Mallinckrodt. Formic acid used for preparation of the liquid chromatography mobile phase was obtained from Fisher Scientific.

C. Test Systems

The test system was defined as the soil, sediment and water fortified with etofenprox and its metabolites α-CO and 4'-OH for determination of recoveries of these analytes by the methods employed. Soil was obtained from a pre-qualification field-collected soil sample (Sample Number LA05-33), collected approximately 35 days prior to application of etofenprox, from Field Test Number 1641-05-434-21E-03 (NY), a field dissipation test site (1). Sediment was obtained from a pre-treatment field-collected soil sample (Sample Number LA05-245), collected approximately 1 day prior to application of etofenprox, from Field Test Number 1642-05-434-21V-05 (AR), an aquatic dissipation test site (2). Water (Sample Number LA05-428) was likewise obtained from Field Test Number 1642-05-434-21V-05 (AR) and was collected prior to any application of etofenprox for purposes of method development and validation (2).

Physical characteristics of soil from the field dissipation site and soil from the aquatic dissipation site associated with the sediment are summarized below:

Soil Source: Field Test Number 1641-05-434-21E-03
Location: New York (NY)
Texture: Loamy Sand (78.8% sand, 14.4% silt, 6.8% clay)
pH: 6.1

Organic Matter: 3.02%
Cation Exchange Capacity: 5.17 meq/100g
Bulk Density: 1.47 g/cc
Water Holding Capacity: 10.62% at 1/3 bar; 4.00% at 15 bar

Soil Source (associated with sediment): Field Test Number 1642-05-434-21V-05
Location: Arkansas (AR)
Texture: Loam (40.0% sand, 44.4% silt, 15.6% clay)
pH: 5.9
Organic Matter: 0.82%
Cation Exchange Capacity: 5.70 meq/100g
Bulk Density: 1.53 g/cc
Water Holding Capacity: 21.46% at 1/3 bar; 5.00% at 15 bar

D. Reference Standard Solution Preparations

Standard solutions, containing etofenprox, α-CO and 4'-OH, were prepared for fortification of control matrix for determination of method recoveries and as calibration standards for the LC/MS/MS instrument. All standard solutions prepared were stored refrigerated when not in use.

Individual primary stock solutions of etofenprox, α-CO and 4'-OH were prepared by accurately weighing approximately 100 mg of each compound using weigh paper and transferring to 100-mL volumetric flasks. Each flask was brought to volume with methanol. Amounts weighed compensated for the purities of each standard such that each solution contained 1.00 mg/mL. A combined standard solution (CS-1), containing 100 µg/mL of each standard, was then prepared by addition of 10.0 mL of each primary stock solution to a 100-mL volumetric flask and dilution to volume with methanol. The 100-µg/mL secondary stock (CS-1) was then serially diluted, 10 mL to 100-mL final volume with methanol, to prepare solutions containing 10.0 (CS-2), 1.00 (CS-3) and 0.100 (CS-4) µg/mL of each standard. Additional solutions were prepared at concentrations of 0.0200 (CS-5) and 0.00200 µg/mL (CS-6) by dilution of 2.00 mL of the 1.00- (CS-3) and 0.100-µg/mL (CS-4) solutions to 100-mL final volume with methanol in 100-mL volumetric flasks, respectively.

Instrument calibration standard solutions, each containing etofenprox, α-CO and 4'-OH, were prepared at concentrations of 0.100, 0.250, 0.500, 0.750, 1.00, 5.00 and 10.0 µg/L and designated ETO-1 through ETO-7, respectively. Calibration standard solutions designated ETO-1 through ETO-5 (0.100 to 1.00 µg/L) were prepared by dilution of aliquots (100 to 1000 µL) of the 0.100-µg/mL secondary stock solution (CS-4) to 100-mL total volume whereas calibration standard solutions ETO-6 and ETO-7 were prepared by dilution of aliquots (500 and 1000 µL) of the 1.00-µg/mL secondary stock solution (CS-3) to 100-mL total volume. Solutions were prepared by dilution of the secondary stock solution aliquots with acetonitrile:water:formic acid (50:50:0.1, v:v:v).

E. Analytical Method

1. Soil and Sediment

The method for analysis of etofenprox, α-CO and 4'-OH in soil and sediment is presented below and schematically in Figure 1.

For each sample, 5.00 g of soil or sediment was weighed into a labeled, 4-ounce, glass, screw cap bottle. Procedural recoveries were fortified using control (untreated) soil or sediment with aliquots of an appropriate combined reference standard solution. Untreated soil or sediment was used for matrix blank samples. For sediment only, approximately 2.5 g of anhydrous sodium sulfate was added to absorb any residual water. To each bottle, 50.0 mL of acetone was added and the sample extracted using ultrasonic disruption for approximately five minutes. For sediment, an approximate 10-mL aliquot of the extract was decanted and centrifuged at approximately 1500 revolutions per minute (rpm) for approximately five minutes. For soil, the entire bottle was centrifuged at approximately 1500 rpm for approximately five minutes.

An appropriate number of Varian Bond Elut® - Si SPE columns (500 mg sorbent, 3 mL) was prepared by rinsing with two column volumes of acetone allowing the second rinse to elute only to the top of the column packing frit. One mL (1.00 mL) of the centrifuged acetone extract was quantitatively transferred to the solid phase extraction column. A slight vacuum was used to elute the sample extract at a flow rate of approximately 1 to 2 drops per second. The eluant was eluted only to the top of the column packing frit. The eluate was collected in a 15-mL glass disposable culture tube. Each column was eluted with an additional 2.0 mL of acetone collecting the eluate in the same collection tube.

Column elates were evaporated to dryness using a gentle stream of nitrogen using a nitrogen evaporator with a bath temperature of approximately 40 to 50°C. The dried extract was reconstituted with 4.00 mL of acetonitrile with mixing followed by addition of 4.00 mL of 0.2% formic acid solution to achieve a final solvent composition of acetonitrile:water:formic acid of 50:50:0.1 (v:v:v). An aliquot of the final reconstituted extract was transferred to an autosampler vial and submitted for LC/MS/MS analysis for quantitation of etofenprox, α-CO and 4'-OH.

2. Water

For purposes of method validation for quantitation of etofenprox, α-CO and 4'-OH in water, the method was applied as presented below and schematically in Figure 2. For field samples of variable volume, changes in this procedure will be implemented as regards aliquot volumes of the dichloromethane (DCM) extraction solvent, incorporation of solvent rinses of the original sample container to remove potentially surface bound residue

and the initial volumes of procedural recoveries to more closely match the volumes of field samples significantly greater than 20 mL.

For purposes of validation, 20.0 mL of water was volumetrically added to a 125-mL separatory funnel. Procedural recoveries were prepared by fortifying control (untreated) water with aliquots of an appropriate combined reference standard solution(s). Untreated water was used for matrix blank samples. Twenty-five mL (25 mL) of DCM was added to the separatory funnel. The sample was shaken with venting for approximately one minute and the phases allowed to separate. The lower DCM phase was drained into an appropriate size round bottom flask. The extraction procedure was repeated with an additional 25 mL of DCM and the DCM phase combined with the first in the round bottom flask. Ten mL (10 mL) of acetone was then added to the round bottom flask to simulate addition of acetone arising from an additional container rinse for field-collected samples.

The combined DCM/acetone extract was rotary evaporated to near dryness at a water bath temperature of approximately 40°C. The residual extract was evaporated to dryness using a gentle stream of nitrogen. The dried extract was reconstituted with 2.00 mL of acetonitrile with mixing followed by addition of 2.00 mL of 0.2% formic acid solution to achieve a final solvent composition of acetonitrile:water:formic acid of 50:50:0.1 (v:v:v). An aliquot of the final reconstituted extract was transferred to an autosampler vial and submitted for LC/MS/MS analysis for quantitation of etofenprox, α-CO and 4'-OH.

3. LC/MS/MS Quantitation

Quantitation of etofenprox, α-CO and 4'-OH was performed using LC/MS/MS. Liquid chromatographic and mass spectral operating parameters are presented in Table 1. The liquid chromatograph was connected to the mass spectrometer through a Valco valve that diverted the first 4 minutes of eluant (post-injection) to waste. 4'-OH was quantitated in negative ion, multiple reaction monitoring (MRM) mode monitoring the transition from 391 to 108 amu. α-CO and etofenprox were quantitated in positive-ion MRM monitoring transitions of 177 to 107 and 359 to 183 amu, respectively.

F. Preparation of Fortified Soil, Sediment and Water

Seven replicates of soil and sediment were fortified at 0.01 mg/Kg and five replicates at 0.100 mg/Kg (of each analyte) with combined standard solutions of etofenprox, α-CO and 4'-OH. Fortified soil replicates, 5-g each, were prepared by fortification of control soil with 500 µL of stock solutions of concentrations of 0.100 (CS-4) and 1.00 µg/mL (CS-3), respectively. Fortified sediment replicates, 5-g each, were prepared by fortification of control sediment with 50 µL of stock solutions of concentrations of 1.00 (CS-3) and 10.0 µg/mL (CS-2), respectively. Two untreated

soil and sediment samples were prepared at the same time to serve as matrix blank samples.

Seven replicates of water were fortified at 0.0500 µg/L and five replicates at 0.500 µg/L (of each analyte) with combined standard solutions of etofenprox, α-CO and 4'-OH. Fortified water replicates, 20-mL each, were prepared by fortification of control water with 500 and 100 µL of stock solutions of concentrations of 0.00200 (CS-6) and 0.100 µg/mL (CS-4), respectively. Two untreated water samples were prepared at the same time to serve as matrix blank samples.

## V. CALCULATIONS

### A. Standard Curves by Linear Regression

Standard curves, for etofenprox, α-CO and 4'-OH for each matrix, were prepared using the peak area responses and known concentrations of the calibration standard solutions injected for each analytical sequence. Standard curves were generated using the linear regression function of Analyst 1.4.1 software applied to the peak area responses and calibration standard solution concentrations. Concentrations of etofenprox, α-CO and 4'-OH were calculated from their peak area responses relative to the linear regression function relating peak area response and concentration. For all analytical sequences, calibration curve plots were prepared with the analyte solution concentration (µg/L) on the abscissa and the respective peak area response on the ordinate. Linear regression was applied to the peak area responses and calibration standard solution concentrations to determine the equation relating peak area and analyte concentration as shown below:

$$A = mC + b$$

where A = peak area (area units)
m = slope of the line (area units/concentration in µg/L)
C = concentration (µg/L)
b = intercept (area units)

### B. Etofenprox, α-CO and 4'-OH Concentrations in Soil and Sediment

Concentrations of etofenprox, α-CO and 4'-OH in soil and sediment (expressed as µg/Kg) were calculated using the following equation and the known values of the intercept (b) and slope (m) from the calibration curve and the measured peak areas (A) of the analyte in the sample extracts:

$$C_{\mu g/Kg} = \left(\frac{A-b}{m}\right)\left(\frac{1\,L}{1000\,mL}\right) \text{ X Dilution Factor}$$

$$\text{where Dilution Factor} = \left(\frac{\text{Extraction Volume (mL)}}{\text{Subsample Mass Extracted (kg)}}\right)\left(\frac{\text{Final Volume (mL)}}{\text{Initial Volume (mL)}}\right)$$

where the Extraction Volume (50.0 mL of acetone) is the quantitative post extraction volume, the Initial Volume (1.00 mL) is the aliquot of the Original Final Volume processed through solid-phase extraction and the Final Volume is the volume of reconstituted sample just prior to LC/MS/MS injection. The Final Volume for control and fortified samples consisted of 8.00 mL of acetonitrile:water:formic acid (50:50:0.1, v:v:v).

Concentrations were then converted to mg/kg for soil and sediment as follows

$$C_{mg/kg} = C_{\mu g/kg} \times \left(\frac{1\,mg}{1000\,\mu g}\right)$$

C. Etofenprox, α-CO and 4'-OH Concentrations in Water

Concentrations of etofenprox, α-CO and 4'-OH in water (expressed as µg/L) were calculated using the following equation and the known values of the intercept (b) and slope (m) from the calibration curve and the measured peak areas (A) of the analyte in the sample extracts:

$$C_{\mu g/L} = \left(\frac{A - b}{m}\right) \times \text{Dilution Factor}$$

$$\text{where Dilution Factor} = \left(\frac{\text{Final Volume (mL)}}{\text{Initial Volume (mL)}}\right)$$

where the Initial Volume is 20.0 mL of water and the Final Volume for control and fortified samples consisted of 4.00 mL of acetonitrile:water:formic acid (50:50:0.1, v:v:v).

D. Recoveries

Recoveries for etofenprox, α-CO and 4'-OH in fortified samples were determined by comparison of the concentration found relative to the nominal concentration added as follows:

$$\text{Percent Recovery} = \frac{\text{Concentration Found}}{\text{Concentration Added}} \times 100\%$$

E. Example Calculation

1. Residue Concentration – Calibration Curve

Linear regression of the calibration standard solution peak areas and their known concentrations, analyzed in the sequence run on September 9, 2005 during method validation for etofenprox in soil, yielded the following results:

Peak Area = (51887.5)(C) + 20.6643, r = 0.9985

Representative chromatograms of a low-level etofenprox calibration standard, high-level etofenprox calibration standard and the resulting etofenprox standard curve derived from the calibration standards injected with this sequence are presented in Figures 3, 4 and 5, respectively. A representative chromatogram of a reagent blank that accompanied this analytical sequence is presented in Figure 6.

2. Residue Concentration – Etofenprox in Soil

Sample Number SVMAS-1, a sample fortified at 0.0100 mg/Kg with etofenprox, was analyzed in this sequence and gave a peak area of 6688.9 units. The chromatogram of Sample Number SVMAS-1 is presented in Figure 7. The accompanying chromatogram of the soil matrix blank from which it was prepared is presented in Figure 8. The etofenprox concentration for this sample, 0.0103 mg etofenprox/Kg was calculated as follows:

$$C_{\mu g/kg} = \left(\frac{6688.9 - 20.6643}{51887.5}\right)\left(\frac{1\,L}{1000\,mL}\right)\left(\frac{50.0\,mL}{0.005\,Kg}\right)\left(\frac{8.00\,mL}{1.00\,mL)}\right)$$

$C_{\mu g/kg}$ = 10.3 µg/Kg

This residue value was then converted to mg/Kg as follows:

$$C_{mg/kg} = C_{\mu g/kg}\,X\left(\frac{1\,mg}{1000\,\mu g}\right) = 0.0103\ mg/Kg$$

3. Procedural Recovery

The percent recovery was calculated as follows:

$$\text{Percent Recovery} = \frac{0.0103\ mg/Kg}{0.0100\ mg/Kg}\ X\ 100 = 103\%$$

Note: Calculations were performed using Analyst Version 1.4.1 software algorithms in full precision mode. Values calculated using the rounded numbers presented may differ slightly.

F. Limits of Detection and Quantitation

The limit of detection (LOD) and quantitation (LOQ) were determined in accordance with procedures presented by the Office of Pesticide Programs in the document titled "Assigning Values to Non-Detected/Non-Quantified Pesticide Residues in Human Health Food Exposure Assessments", March 23, 2000 (3). The method requires the analysis of seven or more control (untreated) samples fortified at the defined/target

LOQ. The standard deviation of the area responses of these samples was determined and the LOD and LOQ were calculated using the following equations:

$LOD = (t_{0.99})(S)$
$LOQ = 3 \times LOD$

where t = the one-tailed t-statistic at the 99% confidence level for n-1 replicates
S = standard deviation of n samples fortified at the defined/target LOQ.

As an example, the standard deviation (S), 0.000294, for the seven replicate etofenprox fortified samples of soil at the target LOQ of 0.0100 mg/Kg was calculated from the recovery values presented in Table 2. For seven replicates, the $t_{0.99}$ value is 3.143 (n - 1 = 6 degrees of freedom). The LOD and LOQ were then calculated as follows:

$LOD = (3.143)(0.000294) = 0.00092$ mg/Kg

$LOQ = 3 \times 0.00092 = 0.0028$ mg/Kg

Table 1. High Performance Liquid Chromatography/Mass Spectrometry Operating Conditions.

| | |
|---|---|
| Instrument: | Hewlett-Packard Series 1100 High Performance Liquid Chromatograph (HPLC) coupled with a Perkin-Elmer SCIEX API 3000 Mass Spectrometer (MS) operated in the multiple reaction monitoring (MRM) mode. |
| Analytical Column: | Phenomenex Luna 5µm C-18 (150 x 3.0 mm, 5 µm particle size) analytical column. |
| Column Oven Temperature: | 40°C |
| Mobile Phase: | A - 0.1% formic acid B - acetonitrile |
| Mobile Phase Gradient: | Time / Percent A / Percent B<br>0.00 / 20.0 / 80.0<br>0.01 / 20.0 / 80.0<br>1.00 / 20.0 / 80.0<br>6.00 / 2.0 / 98.0<br>10.0 / 2.0 / 98.0<br>10.1 / 20.0 / 80.0<br>15.0 / 20.0 / 80.0 |
| Flow Rate: | 250 µL/minute |
| Injection Volume: | 100 µl |
| Ion Source: | TurboIon Spray |
| Period 1 Parameter Table: (4'-OH Quantitation) | NEB: 12.00 DP: -56.00<br>CUR: 9.00 FP: -200.00<br>CAD: 7.00 EP: -10.00<br>TEM: 450.00 CE: -54.00<br>IS: -4500.00 CXP: -17.00 |
| Period 2 Parameter Table: (α-CO and Etofenprox Quantitation) | NEB: 12.00 DP: 51.00<br>CUR: 9.00 FP: 250.00<br>CAD: 7.00 EP: 10.00<br>TEM: 450.00 CE: 30.00<br>IS: 5500.00 CXP: 18.00 |
| Monitored Transitions: | |
| 4'-OH | 391 → 108 (dwell time 500 msec) |
| α-CO | 177 → 107 (dwell time 500 msec) |
| Etofenprox | 359 → 183 (dwell time 500 msec) |
| Approximate Retention Times: | |
| 4'-OH | 6.0 – 6.3 minutes |
| α-CO | 8.8 – 9.1 minutes |
| Etofenprox | 9.5 – 9.8 minutes |

Figure 1. Method Outline for the Analyses of Etofenprox, α-CO and 4'-OH in Soil and Sediment.

Prepare samples and procedural recoveries by weighing 5.00 g of soil or sediment into labeled 4-ounce, glass, screw cap bottles. Prepare procedural recoveries by fortification of control soil or sediment with an appropriate reference standard solution.

↓

For sediment only, add approximately 2.5 grams of anhydrous sodium sulfate to each sample.

↓

Add 50.0 mL of acetone to each bottle. Extract the samples using ultrasonic disruption for approximately five minutes.

↓

For sediment, decant an approximate 10-mL aliquot and centrifuge for approximately five minutes at approximately 1500 rpm. For soil, centrifuge the entire bottle for approximately five minutes at approximately 1500 rpm

↓

Prepare an appropriate number of Varian Bond-Elut® - Si SPE columns (500 mg sorbent, 3 mL) by rinsing with two column volumes of acetone allowing the rinses to elute only to the top of the packing frit.

↓

Quantitatively transfer 1.00 mL of the centrifuged extracts to the solid phase column. Use a slight vacuum to elute samples onto the SPE columns at a flow of approximately 1 to 2 drops/second. Elute only to the top of the packing frit. Collect the eluate in a 15-mL glass disposable culture tube. Elute each column with an additional 2.0 mL of acetone collecting the eluate in the same tube.

↓

Evaporate the acetone extracts to dryness using a nitrogen evaporator with a bath temperature of approximately 40 to 50°C.

↓

Reconstitute the dried extracts initially in 4.00 mL of acetonitrile. Mix well. Add 4.00 mL of 0.2% formic acid solution to achieve a final solvent composition of acetonitrile: water: formic acid of 50:50:0.1 (v:v:v). Mix well.

↓

Volumetrically dilute the extracts further using acetonitrile: water: formic acid solution (50:50:0.1, v:v:v), if required.

↓

Transfer an aliquot of each extract to an autosampler vial and submit for LC/MS/MS quantitation of etofenprox, α-CO and 4'-OH.

Figure 2. Method Outline for the Analyses of Etofenprox, α-CO and 4'-OH in Water.

For purposes of method validation, the following method was used. Changes in the method must be implemented for field samples of variable or unknown volumes with regard to volumes of extraction solvent, incorporation of a solvent rinse(s) of the original sample container, and volumes of accompanying procedural recoveries to more closely match volumes of field samples.

Prepare samples and procedural recoveries by adding 20 mL of water volumetrically to 125-mL separatory funnels. Fortify each procedural recovery by fortification of control water with appropriate reference standard solutions.

↓

Add 25 mL of dichloromethane (DCM) to each separatory funnel. Shake with venting for approximately one minute and allow the phases to separate. Drain the lower DCM phase into a round bottom flask. Repeat the extraction procedure with an additional 25 mL of DCM combining the DCM phase with the first in the round bottom flask.

↓

Add 10 mL of acetone to the round bottom flask to simulate the use of acetone as a rinse solvent for the original sample container for field-collected samples (see Note below).

Note: Use the DCM extraction solvent aliquots to perform rinses of the original sample container for field collected sample prior to use as the extraction solvent. Additionally, rinse the original sample container with 10 to 50 mL of acetone and add directly to the DCM in the round bottom flask.

↓

Rotary evaporate the DCM/acetone extracts to near dryness with a water bath temperature of approximately 40°C. Evaporate the residual extract under a gentle stream of dry nitrogen.

↓

Reconstitute the dried extracts initially in 2.00 mL of acetonitrile. Mix well. Add 2.00 mL of 0.2% formic acid solution to achieve a final solvent composition of acetonitrile: water: formic acid of 50:50:0.1 (v:v:v). Mix well

↓

Transfer an aliquot of each extract to an autosampler vial and submit for LC/MS/MS quantitation of etofenprox, α-CO and 4'-OH.